BIOMETRIC CHARACTERISTICS OF FLOWERS, FRUITS AND SEEDS OF AMERICAN POKEWEED (*Phytolacca americana* L.)

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Abstract. The economic and scientific interest in *Phytolacca americana* L. (American pokeweed) plants has increased in recent years due to its good adaptation to the European climate and high pharmacological potential. The purpose of this study was to evaluate the bio-morphological traits of different parts of *P. americana* plants growing wild in the Slovak Republic and propagated by seeds in the Republic of Moldova. Biometric assessments were performed using AxioVision software applied to microscopic images of American pokeweed buds, flowers, stamens and berries. The duration of phases for plant development in the first year of plant growing from seeds were determined. The duration of the vegetation cycle of American pokeweed from germination of seeds to winter dormancy was 160-210 days. In cultivated plants, the racemes were longer (145.71 mm) and heavier (91.97 g), and the weight of berries harvested from one raceme was 3-4 times greater than berries from a raceme of wild American pokeweed. A direct correlation was found between the length of the racemes and the number of berries per raceme of American pokeweed from both countries. Pearson correlation coefficients were 0.7125 and 0.7015 respectively, for the samples from the Slovak Republic and the Republic of Moldova. The height, diameter, diameter/height ratio of bud and berries, as well as the length of stamens, diameter of flowers and length and height of seeds were measured. Taking into account the productivity of this plant, which yields 250-550 seeds per raceme, and the possibility of its multiplication by roots, technologies must be developed for using various parts of this plant for the benefit of humans.

Keywords: American pokeweed, bio-morphological characteristic, flower, fruit, seed.

Rezumat. Caracteristici biometrice ale florilor, fructelor și semințlor de cârmâz (*Phytolacca americana* L.). Interesul economic și științific pentru planta *Phytolacca americana* L. (cârmâz) a crescut în ultimii ani datorită bunei adaptări la clima europeană și a potențialului farmacologic ridicat. Scopul acestui studiu a fost de a evalua caracteristicile biomorfologice ale diferitelor părți ale plantelor *P. americana* care cresc sălbatic în Republica Slovacă și înmulțite prin semințe în Republica Moldova. Evaluările biometrice au fost efectuate folosind software-ul AxioVision aplicat la imagini microscopice ale mugurilor, florilor, staminelor și fructelor de cârmâz. A fost determinată durata fazelor de dezvoltare a plantelor în primul an de creștere din semințe. Durata sezonului de creștere a cârmâzului de la germinarea semințelor până la repausul de iarnă a fost de 160-210 zile. La plantele cultivate, ciorchinii au fost mai lungi (145,71 mm) și mai grei (91,97 g), iar masa de pomușoare colectate dintr-un ciorchin a fost de 3-4 ori mai mare decât la pomușoare dintr-un ciorchin de cârmâz sălbatic. S-a găsit o corelație directă între lungimea ciorchinilor și numărul de pomușoare de pe un ciorchin în cârmâzul din ambele țări. Coeficienții de corelație Pearson au fost de 0,7125 și 0,7015, respectiv pentru eșantioanele din Republica Slovacă și Republica Moldova. Au fost măsurate înălțimea, diametrul, raportul dintre diametrul și înălțimea mugurilor și pomușoarelor, precum și lungimea staminelor, diametrul florilor, lungimea și înălțimea semințelor. Ținând cont de productivitatea acestei plante, care produce 250-550 de semințe pe un ciorchin, și de posibilitatea înmulțirii ei prin rădăcini; este necesar de a dezvolta tehnologii pentru utilizarea diferitelor părți ale acestei plante în beneficiul oamenilor.

Cuvinte cheie: cârmâz, caracteristică bio-morfologică, floare, fruct, sămânță.

INTRODUCTION

Phytolacca americana L. 1753, also known as *P. decandra* L. 1763, *P. vulgaris* Crantz 1766; (*P. esculentum*, *P. radix*, *P. acinosa*) and common names of American pokeweed, common pokeweed, pokeberry, poke, Virginian pokeweed, scoke, pocan, garget, pigeonberry, inkberry, American nightshade, is native to the eastern part of North America (BALOCH & JUHASZ, 2008). Approximately half of the non-native species on the territory of European country come from the American continent, and American pokeweed is no exception. *P. americana* L. is a popular medicinal herb in Korea, Japan, and China (HAN et al., 2020; BAILLY, 2021). The powder from the dried roots of this plant was included in the Chinese Pharmacopeia as allopathic medicine for carbuncle treatment, and for purgative, salve, bronchodilator and diuretic preparations. Various pharmacological effects (anti-inflammatory, antifungal, antiproliferative activity) were observed for the extracts of root and aerial parts of *P. americana* (SALERI et al., 2017; BAILLY, 2021). The leaves and seeds of *P. americana* produce pokeweed antiviral protein (PAP) with increased antiviral and antifungal activities (TUMER et al., 2000; DOMASHEVSKIY & GOSS, 2015). Additionally, the extracts from *P. americana* berries are a promising source of bio-colorant (BAAKA et al., 2019). American pokeweed plants reproduce by seeds, root cuttings and micropropagation using stem cuttings or other organs of plant (FLOREA & DONEA, 2010; EL-MINISY et al., 2017; TRUNJARUEN et al. 2022, STRGULC et al., 2023). A proper adaptation and large distributions of American pokeweed in the neighbouring countries of the Republic of Moldova such as Romania (DOROFTEI & COVALIOV, 2013) and Ukraine (ZUZA & HUTIANSKYI, 2020; MOSYAKIN & MOSYAKIN, 2021), and in neighbouring countries of the Slovak Republic such as Hungary (BALOCH & JUHASZ, 2008) and Austria (FOLLAK et al., 2022) was reported. Taking into account the medicinal potential and economic impact of the American pokeweed, the aim of this research was the biometric evaluation of morphological traits of different parts of *P. americana* plants growing in Slovakia and the Republic of Moldova.

MATERIAL AND METHODS

Plant materials. This research of morpho-biological features of pokeweed was carried out on plants wildly growing in the Slovak Republic (Nitra) and propagated by seeds in the Republic of Moldova (Chisinau). The pokeweed seeds were sown for germination directly in the field. Planting density was 80 cm between rows and 50 cm between plants.

The bio-morphological characteristics of American pokeweed were studied using microscopic images of buds, flowers, stamens and berries obtained by the Zeiss Discovery V20 microscope (Germany) and the AxioVision software (release 4.8.2. SP2).

The software package Statgraphics Plus 5.0 was used for statistical analysis. The ANOVA test was applied for variance analysis of bio-morphological characters, and the Student test for the assessment of statistically significant differences between treatments (RAUDONIUS, 2017).

RESULTS

55 days after the sowing date, the first seedlings growing in the field conditions were observed. Seedlings were represented by a small orthotropic shoot, 2-3 cm long with 3-4 leaves and weakly expressed internodes. The young generative plants had one leafy orthotropic stem with length of 40-50 cm and 2-4 side branches (Photo 1a). The stems are straight, thick, juicy, green at the beginning of growth and reddish as they grow older. The leaves were of different sizes (length was from 10 to 30 cm), which varied depending on the leaf position on the stem. The leaves of American pokeweed are green, oblong or ovate-lanceolate in shape, and sharp at the apex (Photo 1b). Damaged leaves exude a specific aroma. At the mid-June, the flowering stage began (Photo 1c), which lasted until the first decade of September. American pokeweed inflorescences are racemose and multi-flowered (Photo 2a, b).

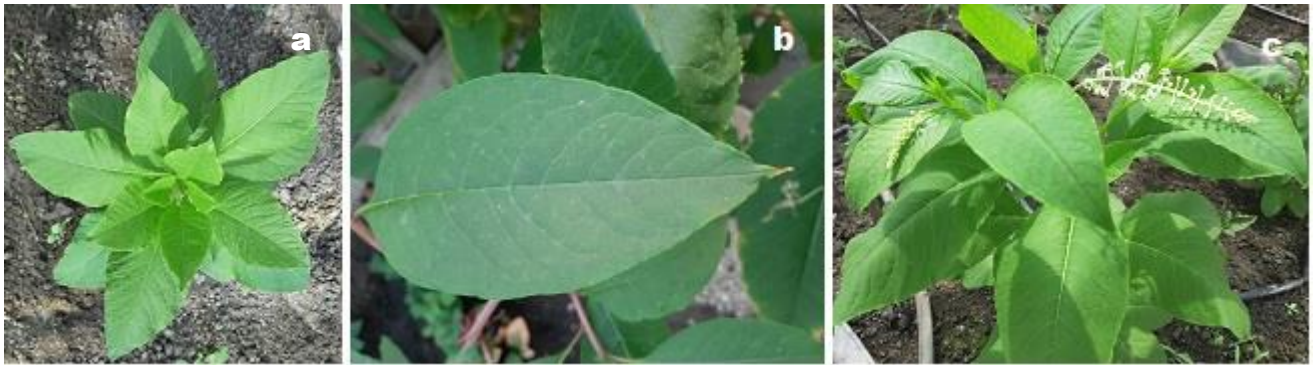

Photo 1. American pokeweed seedlings propagated by seeds in field (original).

Photo 2. American pokeweed inflorescences at the beginning of flowering (a); flowers and first berries (b); berries in the initial stage of ripening (c); ripened fruit (d) (original).

The colour of the petals should be white, green and pink to red, they are small (diameter 6.25±0.53 mm), bisexual. The inflorescences are inclined under the heaviness of initially light green (Photo 2c) and then reddish-purple berries (Photo 2d). Thus, the annual plants of *P. americana* showed the following duration of growth stages: 55 days for seeds germination, 25-35 days of active growth of stem, branch, and leaf areas, 55-60 days of flowering and 50-60 days of fruits ripening. Flowers and fruits were presented simultaneously at different stages of development. The vegetation cycle persisted from March to November.

The bio-morphological characteristics of American pokeweed racemes and berries harvested in 2023 from both countries – the Slovak Republic and Republic of Moldova – were determined (Table 1). A wide variation of determined characteristics was found in samples of both wild growing and cultivated American pokeweed. The racemes of cultivated American pokeweed were longer and heavier, and the weight of berries harvested from one raceme was 3-4 times greater than berries from raceme of wild American pokeweed (Table 1). However, on average, the same number of berries per one raceme was collected from plants growing in different conditions and countries. At the same time, aThe positive correlation was found between length of racemes and number of berries per raceme of pokeweed from both countries. Pearson correlation coefficients were 0.7125 and 0.7015 respectively, for samples from Slovakia and the Republic of Moldova.

One berry contained from 6 to 10 seeds, on average 8.10±1.24 seeds, but the weight of one seed depended on the growth conditions. Thus, the weight of one seed collected from a cultivated American pokeweed plant was 9.11±046 mg, which is 1.5 times more that the weight of one seed from wild plants (Table 1). The weight of seeds predetermines the viability of seeds and the amount of reserve substances that will be used for their further germination.

Table 1. Bio-morphological traits of *P. americana* fruits.

Characteristic	**Length of raceme, mm**	**Quantity per one raceme**		**Weight**				
		Berry	**Seed**	**Whole raceme, g**	**Berries from one raceme, g**	**Raceme without berries, g**	**All seeds from one raceme, g**	**One seed, mg**
P. americana from the Slovak Republic								
min	80.0	40	263	10.70	9.81	0.46	1.60	3.67
max	145.0	65	536	18.40	15.85	1.41	3.68	7.99
average	104.0	49.90	408.70	13.79	12.23	0.91	2.69	6.62
standard deviation	26.85	8.53	71.27	2.32	1.89	0.32	0.67	1.32
variance	721.11	72.77	5089.34	5.37	3.57	0.10	0.45	1.74
P. americana from the Republic of Moldova								
min	80.00	28	252	35.11	28.08	5.70	2.32	8.70
max	175.0	61	549	157,00	66.09	122.96	5.73	10.44
average	145.71	50.00	450.00	91.97	39.77	54.01	4.16	9.11
standard deviation	24.07	11.64	104.76	51.44	11.59	52.66	1.09	0.46
variance	615.35	135.5	2286.9	2811.37	142.66	2945.96	1.18	0.21

Microscopic images of bud, flowers, stamens, green and ripe berries were taken (Photo 3). The buds of American pokeweed have a peony shape of uniform green colour, and form a raceme of inflorescences up to 8-17 cm long (Photo 3a, b). The biometric analysis of American pokeweed buds evaluated that their dimensions were as follows: height 2.20±0.18 mm, diameter 2.45±0.16 mm and diameter-to-height ratio 1.11±0.06 (Table 2). A high positive correlation was found between height and diameter of buds. The Pearson coefficient of correlation was equal to 0.7970. Therefore, the data variation was not significant. The flower consists of five petals, which are separate and not fused (Photo 3c) and the diameter of flowers varied from 5.41 to 7.24 mm (Table 1). The petals of the flower do not have folds or plaits. The number of stamens was 10 and their length in average was 1.84±0.24 mm (Photo 3d, Table 2). The size of green berries inside flowers (Photo 1c; Table 2) was 2.01±0.06 mm in diameter, and, during growth, it reached 6.31±0.82 mm (Table 2). The green berry is round with slightly pronounced sections, and ten of them were also found (Photo 3e). The sizes of ripe berries were measured using pictures from both berries in section and front of view (Photo 3f). Ripe pokeweed berries are glossy and dark purple (purple-black) color, their diameter ranges from 9.40 to 11.71 mm (Table 2). The shape of pokeweed berries is ellipsoidal and the ratio of diameter to height of our samples was 1.40±0.06.

The seeds of American pokeweed are black, reniform-orbicular, glossy and smooth (Photo 3g). The size of seeds collected in both countries, Slovakia and the Republic of Moldova, did not differ; the height was 3.24±0.15 cm and 3.03±0.21 cm; and the width was 2.94±0.23 cm and 2.96±0.28 cm, respectively (Table 3).

Thus, the bio-morphological characteristics of *P.americana* plants growing wild in the Slovak Republic and propagated by seeds in the Republic of Moldova differed significantly in the following traits: raceme length, weight of one raceme, weight of berries per one raceme and weight of one seed. The number of berries per raceme, the number of seeds per one berry and the biometrical characteristics of seeds were identical for samples harvested from both countries.

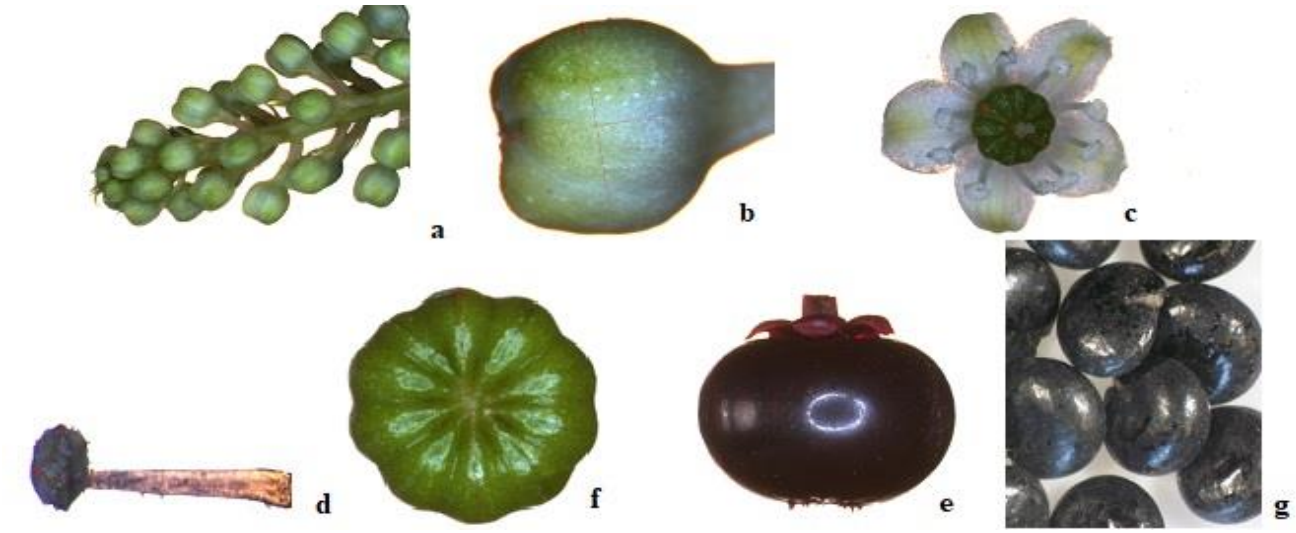

Photo 3. Scan of a microscope image of *P. americana* raceme with flower bud (a), bud (b), flower (c); stamen (d); green berry (e); ripe berry of front view (f), seeds (g) (original).

Table 2. Biometrical characteristics of buds, stamens, flowers and berries of *P. americana*.

Characteristic	Bud			Stamen	Flower	Berry in flower	Green berry	Ripe berry		
	height, mm	diameter, mm	ratio diameter/ height	length, mm	diameter, mm	diameter, mm	diameter, mm	diameter, mm	height, mm	ratio diameter/ height
min	1.88	1.96	1	1.38	5.41	1.92	4.59	9.71	6.36	1.29
max	2.46	2.68	1.26	2.24	7.24	2.10	7.41	11.71	8.66	1.55
average	2.20	2.45	1.11	1.84	6.25	2.01	6.31	10.47	7.51	1.40
standard deviation	0.18	0.16	0.06	0.24	0.53	0.06	0.82	0.51	0.55	0.06
variation	0.03	0.03	0.003	0.06	0.28	0.004	0.68	0.26	0.30	0.004

Table 3. Biometrical characteristics of American pokeweed seeds of different origin.

Characteristics	Slovak Republic		Republic of Moldova	
	Height, cm	Width, cm	Height, cm	Width, cm
min	2.90	2.07	2.70	2.01
max	3.56	3.24	3.52	3.33
average	3.24	2.94	3.03	2.96
standard deviation	0.15	0.23	0.21	0.28
variation	0.02	0.08	0.04	0.08

DISCUSSIONS

Long-term studies on the introduction of the *P. americana* plant by means of seeds have shown that the growing season of annual plants from germination to winter dormancy lasts 160-210 days (FLOREA & DONEA, 2010; IVANOVA et al., 2020). In the field, the American pokeweed seeds germinated on the 55th day. As for American pokeweed seeds, tested in laboratory conditions, 47% of them germinated and 38% developed unfolded cotyledons in 42 days (STRGULC et al., 2023).

In our experiments, the flowering stages began on the 80th day from the date of seed sowing. Therefore, WOLFE-BELLIN et al. (2006) reported that the diurnal and nocturnal temperature could have influenced the beginning of the flowering stage. The plants grown at higher nocturnal temperatures flowered 1.5 days earlier.

As shown in the present study, the stamens of American pokeweed are often arranged in pairs. Previously, it was described (RONSE DECRAENE et al., 1997), that the stamens of the first, second, and third pair arise simultaneously as independent entities. The stamens of the third pair occasionally arise sequentially and are displaced in relation to the third sepal. Stamens opposite sepals 4 and 5 follow sequentially. An analogical distribution of stamens against petals was also established in our study. RONSE DECRAENE et al., (1997) claimed that the delayed plastochron of the stamens opposite sepal 3 indicates that the pentamerous flower has been derived from a trimerous precursor.

Our earlier determination of bio-morphological characteristics of pokeweed fruits is in good concordance with the presented data (IVANOVA et al., 2021). The length of racemes was within 15.00±1.85cm, the weight of berries from one raceme in average was 40.68±3.27g. A direct positive correlation (Pearson coefficient 0.9920) between the length of

the raceme and the weight of berries per raceme was found (IVANOVA et al., 2021). Thus, the determined bio-morphological characteristics of *P. americana plants* growing wildly under the pedoclimatic conditions of the Slovak Republic and reproduced from seeds in the Republic of Moldova were similar to those reported by other scientists (BALOGH & JUHASZ, 2008; IVANOVA et al., 2021).

Previous studies on the introduction of *P. americana* in the Republic of Moldova (FLOREA & DONEA, 2010) led to the conclusion that there is a direct correlation between biometric characteristics, such as the height and mass of the generative stem, the number of leaves, inflorescences and fruits on it. The correlation structure of these characteristics having a big proportion of close positive ties could indicate a certain autonomy of the relevant organs against environmental factors. The strongest correlation was revealed between the height of the generative stems and other studied characteristics of productivity. One generative stem, 1.2-2.6m tall, had from 16 to 39 inflorescences and produced 696-2083 berries (FLOREA & DONEA, 2010). Three-year-old plants of *P. americana* had more than 100 inflorescences. Each inflorescence contains about 50 berries, and one berry - 8-9 seeds; consequently this shrub of American pokeweed can produce over 40 000 seeds annually. Taking into account the productivity of the *P. americana* plant, its good adaptation to the European climate and its possibility of multiplication by seeds and roots, technologies should be developed for using various extracts from leaves and roots as antimicrobial drugs (BAILLY, 2021) and berries extract as a natural colorant (BAAKA et al., 2019).

CONCLUSIONS

The bio-morphological characteristics of *P. americana* plants growing wild in the Slovak Republic and propagated by means of seeds in the Republic of Moldova differed significantly in the following traits: raceme length, weight of one raceme, weight of berries per one raceme and weight of one seed. The number of berries per raceme, the number of seeds per one berry and the biometrical characteristics of seeds were identical for samples harvested from both countries. Future research should be focused on the rational use of various parts of the American pokeweed for human benefit.

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